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Specifications, Physical Properties and Methods of Analysis for Jojoba Oil

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Introduction

Jojoba oil is composed almost entirely of liquid wax esters. Because growing conditions, harvesting and storage treatment can affect the composition of these waxes, standard specifications and methods of analysis are essential in order that all providers and users of jojoba oil can agree on the state of the product. Alteration by chemical reactions, especially hydrogenation, gives a new set of products with different properties that need to be defined. The purpose of this chapter is to detail the methods that are commonly used to evaluate jojoba oil and the specifications that have been developed so far for the commercial oil. First, we describe the industry standards as provided by the suppliers themselves and the ways in which these standards can be determined. In the second part, we discuss the methods that have been used to separate and to analyze the wax esters in order to quantify them by chain length plus tehniques that have been developed to determine the relative amounts of isomers within each chain length. These analyses are necessary when the precise composition of a particular oil is needed for purposes such as plant breeding, identification of atypical oils or for detection of adulteration.

Specifications for Jojoba Oil

To date, industry-wide specifications governing the sale of jojoba wax esters are still being developed and standardized. Jojoba oil is commercially available in at least three grades: natural, refined and modified. Needs of the end user will determine the grade that he should purchase (1).

Natural. Jojoba wax esters are recovered from seed by pressing with expellers. This is repeated several times to obtain as much oil as possible. With each pressing, the wax esters increase in color, free fatty acids,

odor and proteinaceous material. Suitable oils are prepared by judicious blending of the various pressings. Premium quality jojoba oil can be obtained by a single pressing and is considered to be equivalent to extra virgin grades of vegetable oils. Single pressed oils have virtually no odor, less than 0.3% free fatty acids and a light yellow color. In addition, single pressing keeps proteinaceous materials, free acids and alcohols, and peroxides at a minimum, thereby assuring maximum oxidative stability.

Some applications, most notably cosmetic formulations, require jojoba oils completely devoid of color, flavor and odor. These oils can be prepared by molecular distillation and are considered natural because no chemicals are used in the process (1).

Refined. With the exception of molecularly distilled oil, any colorless product is catagorized as refined. Color reduction may be achieved through adsorptive bleaching with activated bleaching earth or charcoal, column refining or caustic soda. Often these treatments are proprietary (1).

Modified. Jojoba can be made more water soluble through ethoxylation, and propoxylation. The melting point of jojoba can be raised through isomerization with catalysts (2). Hydrogenation of jojoba produces a solid, wax-like product similar to beeswax and candelilla waxes and bleaching of hydrogenated liquid jojoba oil gives a product with a wide range of melting points and hardness characteristics (1).

Quality Factors

Common quality factors for jojoba oil include color, odor, acid value, saponification number, iodine value and peroxide value. Table 1 shows the values or descriptions for a variety of oils as provided by three commercial suppliers, together with the methods by which they can be determined. The Official and Tentative Methods of the American Oil Chemists' Society (3) are preferred where applicable. It is clear that these suppliers are in good agreement for the parameters mentioned and thus, they can be inferred as industry standards.

The Gardner color scale originally intended to measure drying oils is commonly used for jojoba oil. Gardner color standards of 1-18 are prepared by mixing ferric and cobalt chloride solutions (4). Unless the jojoba sample is clear, it must be filtered through paper at 25 C before the color can be measured. The clear oil is placed in a sample tube and the color is determined by visual matching.

Acid value is defined as the mg of KOH necessary to neutralize the free acids in 1 g of sample. This parameter is determined by titration

TABLE 1

Specifications for Jojoba Oil

Company A		
Characteristic	Natural	Refined
Visual	Golden yellow	Decolorized
Color-Gardner	9 max	2 max
Acid value mg KOH/g	0.7 max	1.0 max
Peroxide value meq/kg	5.0 max	1.0 max
Thin-Layer chromatography	Representative	Representative

Company B			
Characteristic	Pure grade	Sonora grade	Refined
Color-Gardner	9 max	8 max	2 max
Odor	Typical fatty	Slightly fatty	Odorless
Acid value	<1.00	<1.00	<1.00
Saponification number	90-95	90-95	90-95
Iodine value	80-85	80-85	80-85
Total plate count	<50/gm	<50/gm	<50/gm
Melting point	7-9 C	7-9 C	7-9 C
Flash point	295 C	295 C	295 C
Fire point	338 C	338 C	338 C

Company C			
Characteristic	Tolerance	Typical	Method*
Appearance, 25 C	Clear and free of foreign material		Visual
Color-Gardner	10 max	9.5	Td-1a-64
Acid value	1.0 max	0.2	Cd-3a-63
Odor	Mild fatty	Slightly fatty	Oganoleptic
Iodine value, Wijs	80-85	83	Cd-1-25
Moisture & volatile, %	0.1 max	nil	Karl-Fischer
Density, lbs/gal	—	7.2	Cc-10a-25
Saponification value	90-95	92	Cd-3-25

Bleached Jojoba Oil Same as for pure, cold-pressed jojoba oil except for:			
Color-Gardner	2 max	1	Td-1a-64

(Continued)

Hydrogenated Jojoba Oil

Company A			
Characteristic	Tolerance	Typical	Method*
Appearance 80 C	Clear and free of foreign material		Visual
Color-Gardner	2 max	1	Td-1a-64
Acid value	1 max	0.2	Cd-3a-63
Odor	Mild, waxy		Organoleptic
Iodine value, Wijs	1 max	0.6	Cd-1-25
Melting point	70 min	71	Mettler
Saponification value	90-95	93	Cd-3-25

Company B			
Characteristic	Isomerized	Blended	Hydrogenated
Color	Light amber	Light amber	White
Odor	Slightly fatty	Slightly.fatty	Odorless
Appearance	Semisolid wax	Semisolid wax	Crystalline
Melting point	28-31 C	48-51 C	70-72 C
Iodine value	80-85	65-75	Less than 2

*Refers to *Official and Tentative Methods of the American Oil Chemists' Society* (3), unless otherwise indicated.
**Blend of fully hydrogenated and isomerized jojoba oils.

(Method Cd-3a-63a). Saponification number is the mg of KOH required to saponify 1 g of oil as prescribed in Method Cd-3-25.

Iodine value is a measure of the unsaturation of the oil and is determined by titration (Method Cd-1-25). Since jojoba oil is essentially composed of dienes (one double bond per fatty alcohol and acyl group), its iodine value is similar to that of oleic acid. Peroxide value is usually defined as the number of milliequivalents of peroxide per kg of sample (or meq/kg). Method Cd-8-53 is used. Unsaponifiable matter in jojoba should be determined by Method CA-6b-53 that was developed for marine oils.

Reactions that deteriorate jojoba oil quality. Two reactions that are likely to occur and that can cause deterioration or loss of quality involve water (hydrolysis), oxygen (oxidation) or both. The hydrolysis reaction can be depicted simply as: wax ester + water → free acids and free alcohols. Enzymatic hydrolysis may occur in the whole jojoba seed prior to processing. Limited data indicate that good quality crude jojoba oil can be prepared from normal seeds having free fatty acid content below

1% and often 0.5% or less. By comparison, good quality crude triglyceride oils like corn, cottonseed, sunflower or soybean show levels of free fatty acids in the same 0.5–1% range. Since jojoba seeds are grown in hot, arid regions, they are not normally exposed to high moisture levels that promote enzymatic hydrolysis. After removal from the seed, crude jojoba oil should not be exposed to moisture, particularly at high temperatures since the solubility of water in wax esters increases markedly with temperature.

During the expelling process, a certain amount of proteinaceous material is carried along with the oil which, in the presence of water, provides an excellent substrate for microbiological reactions. Some processors pasteurize cold-pressed jojoba oil to reduce the problem of microbiological contamination.

Oxidative deterioration results from the reaction of fatty acids and alcohols with molecular oxygen according to the following scheme (5):

The breakdown of fat hydroperoxides yields a myriad of short-chain products which are responsible for odor or flavor traits. Cosmetic and pharmaceutical uses require bland oils. Thus, minimizing oxidation becomes an important factor in the handling and processing of jojoba oils.

The peroxide value of an oil can be used as a measure of its relative oxidation state. Unlike triglyceride oils where the peroxide value of good quality crude oils is usually near zero, crude jojoba oil appears to have titratable amounts of peroxides at the time of production. Studies of the oxidative stability of jojoba wax esters reported by Kampf et al. (6) indicate that crude jojoba contains a natural antioxidant that the authors postulated to be an allylic derivative of hydroxytoluene. Other workers have suggested that the natural antioxidant is not a tocopherol (7), such as are traditionally regarded as natural antioxidants in vegetable oils.

Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), when added (at 0.02% by weight) to bleached jojoba oil, markedly improve its oxidative stability. Bleached jojoba wax is stable toward oxidation when stored in the dark at ambient temperature but, in the presence of light and air, it reaches a peroxide value of 70 meq/kg in 7 weeks. These results emphasize the importance of not exposing fully refined jojoba products to light and oxygen.

Physical Properties

Refractive index, density, viscosity, dielectric constant, specific conduc-

TABLE 2

Physical Properties of Jojoba Oil (8)

Temp C	Ref index n	Temp C	Density q/ml	Temp C	Viscosity cp	Temp C	Sp cond mho/cm	Dielec Const ϵ
25.2	1.4645	25.6	0.8656	25.6	35.2	27.0	8.86×10^{-13}	2.680
30.0	1.4625	38.0	0.8572	30.5	29.6	32.5	6.02×10^{-12}	2.886
35.0	1.4610	46.6	0.8514	35.7	24.9	46.0	8.80×10^{-12}	2.848
40.0	1.4590	55.6	0.8459	40.7	21.0	60.2	1.24×10^{-11}	2.840
45.1	1.4570	64.6	0.8393	45.8	18.0	74.6	1.66×10^{-11}	2.789
50.0	1.4550	74.9	0.8330	51.0	15.6	84.6	2.36×10^{-11}	2.783
55.2	1.4535	84.4	0.8266	55.6	13.7	98.6	4.60×10^{-11}	2.760
60.1	1.4520	97.7	0.8181	60.8	11.9	107.0	5.29×10^{-11}	2.726
65.3	1.4500	106.2	0.8123	65.6	10.6	122.8	9.96×10^{-11}	2.703
70.4	1.4489	113.5	0.8076	75.4	8.5	140.0	11.52×10^{-11}	2.646
75.6	1.4465	123.6	0.8008	85.6	6.9	—	—	—
—	—	129.4	0.7971	101.5	5.2	—	—	—
—	—	145.0	0.7872	110.1	4.5	—	—	—
—	—	148.6	0.7848	—	—	—	—	—
—	—	164.8	0.7743	—	—	—	—	—

tivity and surface tension data for jojoba have been reported in detail by Wisniak and Liberman (8). These values are shown in Table 2. Refractive index measurements over the range 25-75 C showed that n = 1.47341 - 0.000360T, where n = refractive index and T = °C. The accuracy was estimated at ±0.00001. Density data determined over the range 25-165 C showed the following relationship: d = 0.8821 -0.000656T, where d = density in gm/ml and T = °C. Absolute viscosity data over the range 25-110 C showed that an Arhenius type equation is followed, where $\cap$ = $0.004995^{(2646/T)}$, with T = °C. The viscosity index of jojoba was reported as 225. The specific conductivity of jojoba is similar to that of oleic acid and the dielectric constant falls in the ranges reported for other fatty materials. The surface tension of jojoba oil determined at 23.5 C has a value of 34 dynes/cm.

Chemical Analysis of Jojoba Oil

Jojoba oil consists of wax esters ranging in chain length of 34-50 carbon atoms (9). The relative proportion of each chain length and the composition of the isomers within a chain length can vary from oil to oil, so that sophisticated techniques are necessary in order to quantify the entire oil. Usually, investigators are primarily concerned with the overall composition by chain length or with the composition of the constituent fatty acids and fatty alcohols (chain length range of 14-26 carbons). These questions are routinely answered by chromatography.

Chromatographic Techniques

Virtually any chromatographic method that has been applied to the analysis of oils can be used with jojoba oil. Modifications of the procedures are sometimes necessary because wax esters differ enough from triglycerides (the matrix for which most analytical procedures were designed) to warrant special steps or systems in order to optimize the results. Fortunately, much of the work that has been done on the analysis of marine oils (the other major source of wax esters) can be almost directly used with jojoba.

Thin-Layer Chromatography (TLC). Figure 1 illustrates a separation of wax esters from other oil constituents by conventional-phase TLC. Here, benzene was used as the development solvent and the wax esters stand out well from the triglycerides of corn and whale oils. Benzene has been implicated as a carcinogen, so its use has been discouraged in many laboratories but it remains an excellent choice for a TLC solvent.

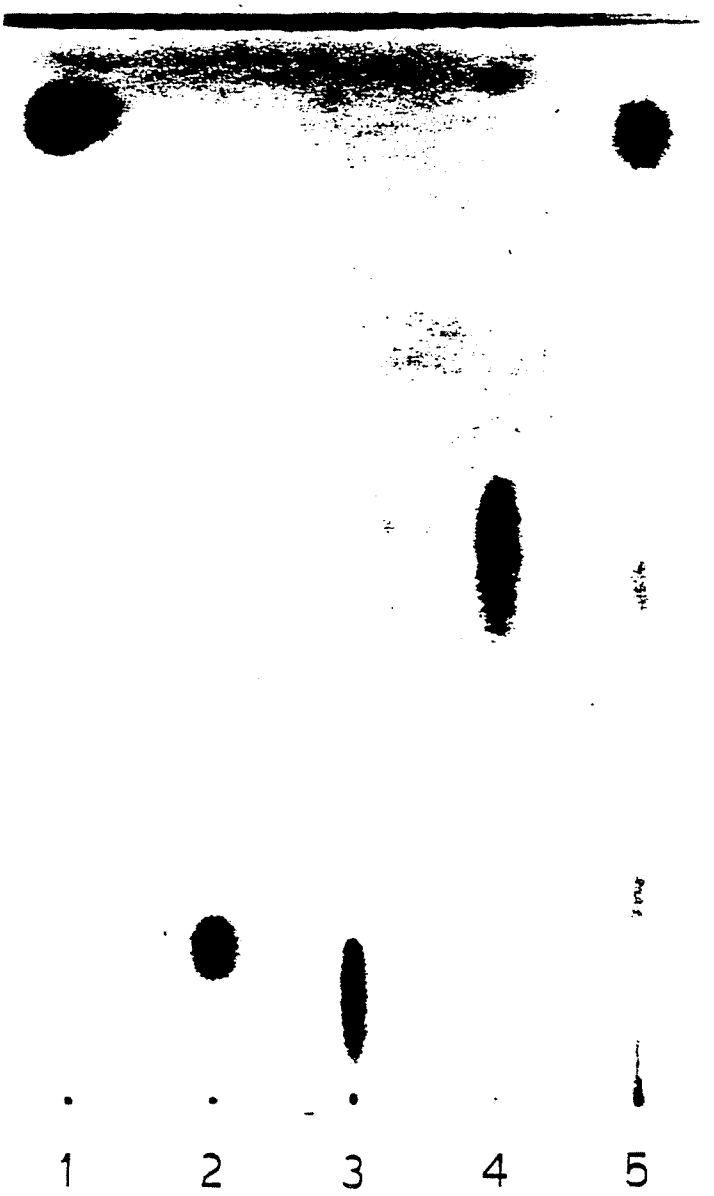

FIG. 1. Thin-layer chromatography on silica (0.25 mm thick). Solvent: benzene. From left to right: 1 = jojoba oil, 2 = oleyl alcohol, 3 = oleic acid, 4 = corn oil, 5 = sperm whale oil.

Toluene gives a similar migration pattern, but the spots are not so distinct. Mixtures of hexane and ether have also been used and, together with plates coated with magnesium hydroxide, enable the separation of wax esters from steryl esters (10). Regardless of the solvent used, TLC should only be performed in a hood. Charring (as shown) or iodine vapor are nearly equally good for visualization of spots.

TLC provides an excellent means of isolating small quantities of wax esters (for any purpose) or to concentrate the wax esters from formulations because the experiment can easily be scaled-up to thicker plates (2 mm of silica) and the separations are nearly as good as on the analytical plates. This is especially of interest to analysts who wish to ascertain whether or not jojoba is present in a complicated matrix such as a cosmetic preparation. One need only dissolve the sample in an appropriate organic solvent (benzene or ether work well) or partition it between the organic phase and water and then use standard, preparative TLC procedures. The partitioning step helps eliminate water-solubles from the matrix making it simpler and more homogeneous. If larger amounts of sample need to be processed, gravity-fed silica columns with elution solvents similar to those used in TLC are very effective.

Anticircular, high-performance TLC (1) was used to analyze cosmetic creams for jojoba oil content. Spots were visualized by phosphomolybdic acid treatment followed by heat and were then scanned at 546 nm by a recording densitometer. In this way, jojoba concentrations of 1-4% in creams and milks were quantitatively measured. Triglyceride content could be obtained simultaneously.

Conventional-phase TLC is not effective for separation of the fatty acids from the alcohols from hydrolyzed jojoba oil (Fig. 1) in one step. A two-plate quantitative TLC method was devised (12) to analyze waxes, fatty esters, alcohol acetates, free acids and free alcohols. Alternatively, the acidified hydrolysis mixture can be treated with diazomethane. This reaction will convert the free acids to methyl esters and thereby make them easily separable from the alcohols. (Methyl or ethyl esters will migrate similarly to the triglycerides under the conditions used in Figure 1. Since methyl esters are often the preferred derivatives for the analysis of fatty acids, the esterification step is not necessarily wasted.

High-Performance Liquid Chromatography (HPLC). The advent of pulse-free pumps and micro-particulate column packings made HPLC an almost indispensable tool for semi-preparative and analytical applications. When used on jojoba oil, a complete separation of the homologous wax esters can be obtained (Fig. 2). If properly calibrated, the refractive index detector can be used as a quantitative tool.

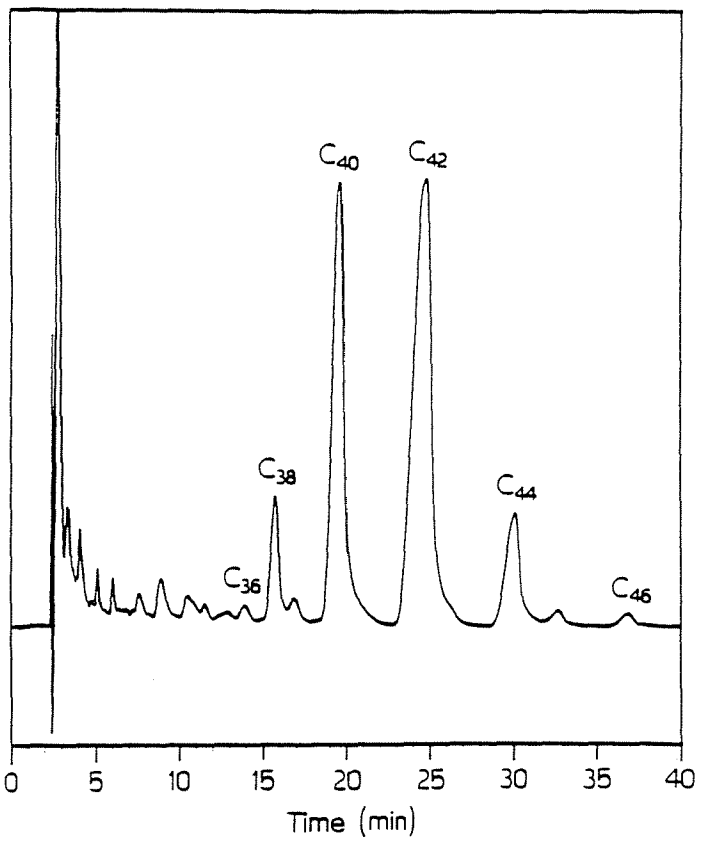

FIG. 2. High-performance liquid chromtography of jojoba oil. Column: 250 × 4.5 mm ODS (octadecylsilane) (Whatman). Solvent: Acetone/acetonitrile (3/1). Detection by differential refractometry.

Even more important, however, is the convenient acquisition of the homologs without harsh treatment. Each "peak" can be collected easily as it emerges from the detector. Removal of the solvent leaves purified wax esters with particular chain lengths. They can then be used for further analytical studies or for biological tests such as feeding experiments. As yet, no system has been developed to separate the isomers within a single chain length by HPLC.

Gas-liquid chromatography (GLC). GLC probably provides more qualitative and quantitative information about the components in oils

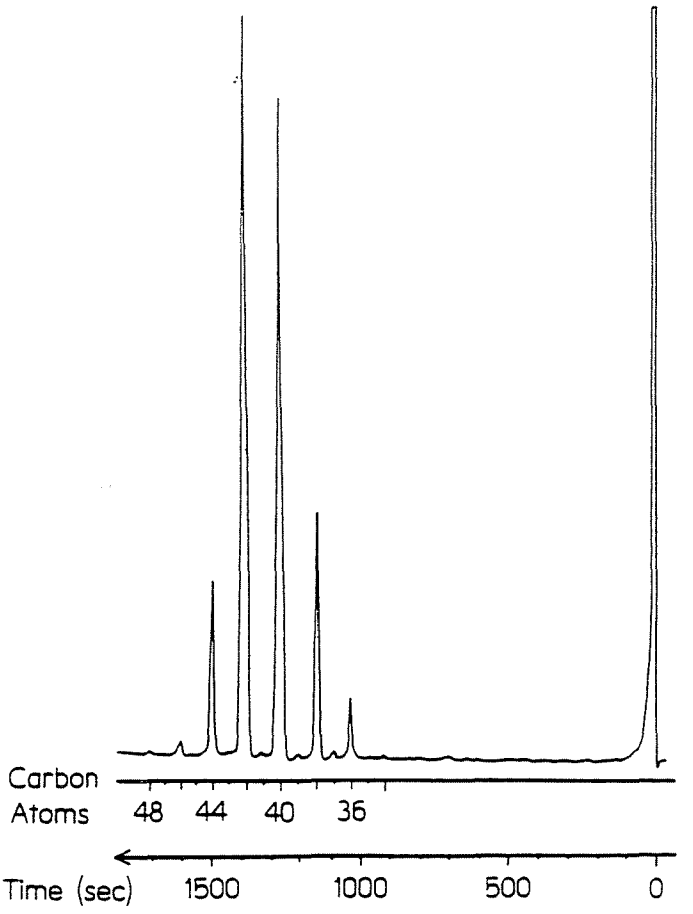

FIG. 3. Gas-liquid chromatography of jojoba oil. Column: OV-1, 3 m × 0.28 mm, with a 0.1 μm film thickness. Injector—325 C; detector—350 C; oven: 1 min at 190 C then programmed to 340 C at 5 C/min. From Graille et al. (12).

than any other single technique. Insofar as jojoba oil is concerned, the percentage of each wax ester homolog plus the overall fatty acid and alcohol compositions can easily be obtained. Miwa (13) first applied GLC to intact jojoba oil and also developed an esterification method that enabled determination of the fatty acids and alcohols in a single GLC run (13). These analyses were made on "packed" columns under conditions that represented the state-of-the-art of gas chromatography at

that time. Although these procedures give accurate compositional data, GLC technology has progressed and now, most analysts prefer capillary or open tubular columns, because the finer separations that are possible can give even more information about the samples.

Graille et al. (12) published the capillary gas chromatogram shown in Figure 3. Excellent resolution of the homologs together with sensitivity and stability are evident. Only one improvement is desirable—the resolution of the isomers within a single chain length. Itabashi and Takagi (14) studied the separation of wax ester isomers by capillary GLC and found that certain shorter-chain (26-32) saturated isomers could be resolved at relatively low column temperatures. These conditions would not be suitable for jojoba oil but, it is certainly possible that further research in this area could lead to significant improvements that eventually would be applicable to longer-chain esters. These authors also determined that wax esters of the same chain length could sometimes be separated by degree of unsaturation. Again, this finding is not directly applicable to unmodified jojoba oil, since essentially all of the compounds are dienes, but partially hydrogenated oils might be analyzed in this way. Rezanka and Podojil (15) used a more efficient capillary column that had ten times as many theoretical plates per meter (for eicosanyl nervonate) than reported by Itabashi and Takagi (14) and illustrated superior chromatograms of samples with chain lengths up to 50 carbon atoms. They also attempted to resolve isomer pairs and unsaturated analogs with limited success. The main separation problem with jojoba is determining the relative amounts of the isomers within a certain chain length, a result that is not achieved with these experiments, but advances in capillary column technology should be monitored because systems that could be applied directly to jojoba are likely to be developed.

Capillary GLC is also the method of choice for analysis of the component alcohols and fatty acids of jojoba oil. Typically, hydrolysis or alcoholysis is first used to break apart the wax ester chains and then proper derivatives are prepared for further GLC work. Both acid (12,13, 16,17) and base (9) catalyzed procedures have been advanced for the hydrolysis step and care must be taken to ensure that the reaction proceeds to completion (12) and that a representative sample of the components is obtained. An example of the capillary GLC of derivatized jojoba oil is shown in Figure 4. Pina and coworkers (18) advanced a derivatization procedure based on reaction of the wax esters with magnesium ethyl bromide that converts the fatty acyl moieties into tertiary alcohols and leaves the original alcohol groups unchanged (other than

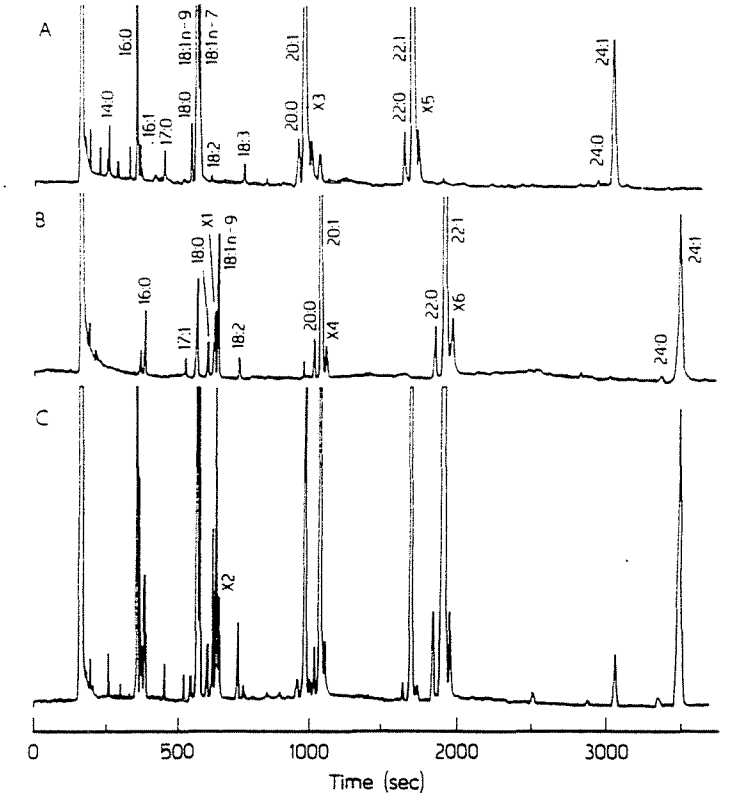

FIG. 4. Gas-liquid chromatography of jojoba oil derivatives. A = fatty acid ethyl esters; B = fatty alcohol acetates; C = fatty ethyl esters = alcohol acetates. column OV-1, 38 m × 0.3 mm, with a 0.1 μm film thickness. Injector—250 C; detector—275 C; column 230 C. From Graille et al. (12).

being hydrolyzed). The reaction results, therefore, in a mixture of primary and tertiary alcohols. Capillary GLC easily resolves the two types of alcohols and distinguishes between analogs of each type giving a one-step derivatization and a one-step chromatographic method (Fig. 5).

Spectrometric Techniques

Infrared, ultraviolet and nuclear magnetic resonance spectra of "pure" jojoba oils look very similar to those of other vegetable oils (if one takes into account the inherent differences due to wax ester and triglyceride structural features). Hence, little compositional information can be

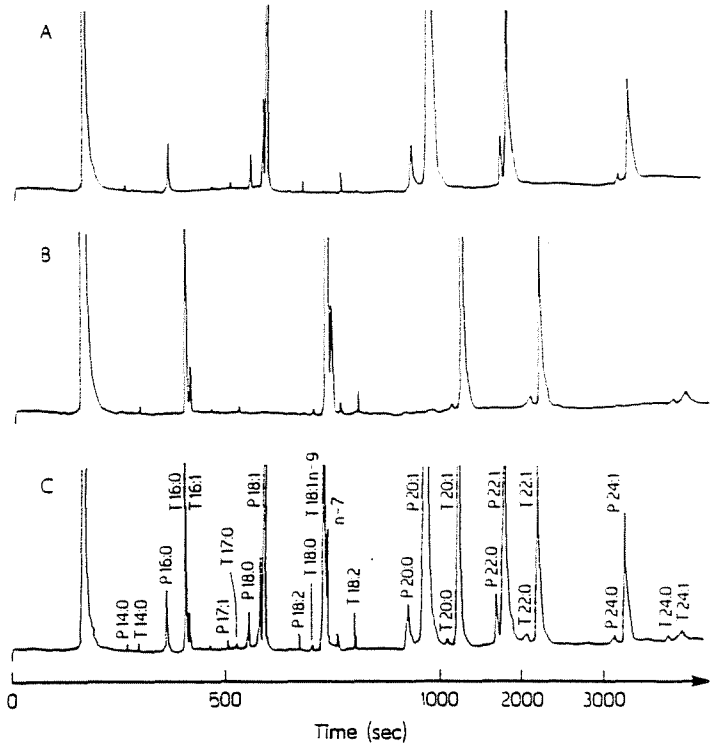

FIG. 5. Gas-liquid chromatography of derived jojoba samples. A = primary fatty alcohols, B = tertiary fatty alcohols, C = primary (P) and tertiary (T) fatty alcohols. Column: Carbowax 20 M, 30 m × 0.317 mm, with a 0.25 μm film thickness. Injector—250 C; detector—275 C; column 200 C or 220 C. From Pina et al. (18).

gained from these spectra and structural details are limited to detection of adulteration or other post-harvest treatment. Mass spectrometry has, however, proven to be a powerful tool in the analysis of wax esters because it can provide definitive quantitation of the isomers. The limiting factor here is the high cost and maintenance of a modern mass spectrometer.

Two approaches have been used to determine wax ester isomers by mass spectrometry, GLC-MS and MS-MS. Prior to the advent of these "hyphenated" techniques, the only possible way to make isomer determinations required that the oil be resolved by chain length (HPLC) and then each fraction be hydrolyzed and analyzed by GLC (9). This procedure makes each oil sample a research project lasting up to two weeks

per analysis, a throughput that is unacceptable if many samples need to be analyzed. Therefore, considerable effort has been made to find an alternative route. The MS-based methods are the only ones so far that have been able to solve the problem.

GLC-MS. Aasen et al. (19) showed that quantitation of isomers was possible by EI-MS (electron-impact mass spectrometry) because, in saturated wax esters, fragmentation of the wax RCOOR′ (where R and R′ represent the alkyl moieties of the acyl and alcohol portions respectively) gives $(RCOOH_2)^+$, $(RCO)^+$ and $(R'H)^+$ as the major ions. They further proved that measurement of the intensities of these ions could provide quantitation of the parent isomers. In an effort to determine the isomer concentrations in whale oil, Spencer (20) used argentation-TLC to divide the waxes by degree of unsaturation and then GLC on a packed column to separate the homologs. MS of the saturated components was quantitated similarly to the methods of Aasen and co-workers (19) and the dienoic compounds could be hydrogenated to saturates and analyzed, but the monoenes gave a further problem because it was desirable to know which moiety (R or R′) was unsaturated. Because the unsaturated portion of the molecule tends to produce greater amounts of ions, it was necessary to use $(RCO-1)^+$ and $(RCO)^+$ to estimate those waxes with an unsaturated acyl group and $(R'-1)^+$ for those where the double bond was in the alcohol portion. This analytical scheme is quite cumbersome and requires great attention to detail in order to achieve good results. Also, GLC and the allied enrichment techniques that are necessary in the GLC-MS interface can discriminate between lower- and higher-boiling compounds and thereby prejudice the answers in favor of the lower-boiling ones. Nonetheless, Rezanka and Podojil (15) used capillary GLC-MS to successfully analyze waxes up to 46 carbon atoms. A thorough investigation of capillary GLC-MS was made by Wakeman and Frew (21), who were able to minimize absorptive losses during GLC-MS. They also used the "softer" ionization technique, chemical ionization (CI), that reduces the amount of secondary ionizations that are not useful for structural analysis (21).

MS-MS. Plattner and Spencer also studied the CI-MS spectra of wax esters (22) and concluded that by using isobutane as the reagent gas they could reduce the secondary fragmentation to virtually nothing in saturated compounds. The only ions of consequence were the protonated molecular ion and the $(RCOOH_2)^+$ ion from the acyl moiety. Unsaturated compounds, however, still had rather complex ionization patterns with the degree of fragmentation directed by the double bond; and they concluded that extensive analyses of standards would be nec-

essary in order to determine the correction factors to be used in calculations of the compositions. What seemed desirable was a method of derivatization of the double bond that would retain the information necessary to determine its location but that would not lead to discriminatory fragmentation.

Spencer and Plattner used tris(triphenylphosphine) chlororhodium(I) catalyst and deuterium to reduce unsaturated wax esters (23). This reduction, which gives very limited exchange between substrate and reagent, essentially adds two deuterium atoms to each double bond and results in saturates with an extra two Daltons (AMU) per double bond. This type of compound is ideally suited for MS analysis. At this time, advances in mass spectrometry had led to the introduction of new instruments that included the triple quadrapole or MS-MS type and that greatly simplified the analysis of deuterated wax esters. The reduced wax ester mixture could be introduced through the solid inlet (thereby eliminating GLC and enricher losses) and ionized by isobutane giving extremely high yields of the protonated molecular ions. These ions were separated by the first mass filter and then passed to the second quadrapole where they were bombarded with argon. This resulted in a disintegration of the ions which gave, under proper conditions, a $(RCOOH_2)^+$ ion from each wax ester. These protonated acyl ions were finally directed through the third mass filter and analyzed. This reaction and analysis scheme proved to be superior to the GLC-based procedures in terms of time and efficacy but requires even more expensive instrumentation.

Recently, Schulten et al. (24) analyzed jojoba wax esters by field desorption (FD) mass spectrometry and reported that jojoba contains a relatively large concentration of heretofore unreported waxes with molecular weights between 1100 and 1300 Daltons. Since this represents a significant departure from traditional jojoba composition, it needs further confirmation (isolated compounds) before it can be determined with surety that the ions that they observed are not anomolies inherent in the FD method.

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